Journal of Medical Virology 50:71–74 (1996)

Identification of GBV-C Hepatitis G RNA in Chronic Hepatitis C Patients

Sabine Schleicher, Ricardo L. Chaves, Thomas Dehmer, Michael Gregor, Georg Hess, and Bertram Flehmig

Department of Medical Virology and Epidemiology of Viral Diseases, Hygiene Institute of the University of Tübingen (S.S., R.L.C., T.D., B.F.); Department of Internal Medicine, University of Tübingen (M.G.), Tübingen; Boehringer Mannheim, Mannheim (G.H.), Germany

Sera from patients with chronic hepatitis C were examined for the presence of GBV-C/HGV RNA by RT-PCR. The amplified products, derived from the 5′ non-coding, NS3, and NS5a regions, were detected in 19 (19%) of the 100 HCV RNA-positive samples. Analysis of GBV-C/HGV prevalence rates revealed that dual infections are related to shared parenteral risk factors. Intravenous drug abuse and multiple transfusions were the factors clearly associated with a simultaneous HCV and GBV-C/HGV infection. Apart from this, patients with dual infections had a statistically significant lower mean age compared to those patients infected solely by HCV. Determination of HCV genotypes involved in GBV-C/HGV coinfection by RFLP analysis showed no correlation between the presence of GBV-C/HGV and a distinct HCV genotype. The study demonstrates that, based on the assessment of risk criteria, GBV-C/HGV is transmitted efficiently parenterally and is frequently linked to hepatitis C coinfection, regardless of HCV genotype.

KEY WORDS: non-A-E hepatitis, hepatitis C virus, coinfection, PCR

INTRODUCTION

Despite the availability of specific and sensitive tests for the detection of the known hepatitis viruses (hepatitis A–E), the etiology of a significant fraction of post-transfusion and community-acquired hepatitis cases has remained unclear [Alter et al., 1989, 1992], suggesting the existence of additional agents which may be transmitted parenterally [Peters et al., 1993; Thiers et al., 1993]

Recently, Simons et al. [1995a] isolated two flavivirus-like genomes in the plasma of tamarins experimentally infected with serum from a surgeon with acute icteric hepatitis. A third related virus, GB virus C (GBV-C), has been identified in serum from patients with idiopathic hepatitis [Simons et al., 1995b; Muerhoff et al., 1995]. In a separate approach, Linnen et al. [1996] discovered another flavivirus-like agent associated with hepatitis, which was provisionally designated hepatitis G virus (HGV). Nucleotide sequence as well as amino acid sequence alignment revealed a general identity between GBV-C and HGV of 86% and 95%, respectively. Based on the high degree of sequence identity, it has been suggested that GBV-C and HGV are independent isolates of the same virus [Zuckerman, 1996].

The GBV-C/HGV genome consists of a positive-stranded RNA molecule containing approximately 9,400 nucleotides and encodes a single large polyprotein which includes protease, helicase, and replicase motifs [Simons et al., 1995b; Leary et al., 1996; Linnen et al., 1996]. The genomic organization is similar to that of the viruses in the Flaviviridae family, with structural regions at the N- terminal end and nonstructural regions at the C-terminal end. The existence of only 26% homology at the amino acid level with the hepatitis C virus (HCV), which belongs to the same family, indicates that GBV-C/HGV is distinct from HCV.

Transmission of the virus by blood or blood products, or other parenteral routes of exposure such as intravenous drug abuse, has been clearly established [Linnen et al., 1996; Nübling and Löwer, 1996]. It may be assumed from these data that many HCV-positive patients suffer from a parallel GBV-C/HGV infection, presumably occurring due to identical risk factors. Our aim was, therefore, to study the prevalence of GBV-C/HGV infection in patients with chronic liver disease who were HCV RNA-positive for one of the various HCV genotypes.

MATERIALS AND METHODS

Sera

One hundred patients (62 men, 38 women), aged 14–67 years (median 41 years), with chronic HCV infection were studied. All patients were anti-HCV-positive by

Accepted for publication May 20, 1996.

Address reprint requests to Prof. Bertram Flehmig, Department of Medical Virology and Epidemiology of Viral Diseases, Hygiene Institute of the University of Tübingen, Silcherstr. 7, 72076 Tübingen, Germany.

second-generation enzyme immunoassay (EIA) (Ortho Diagnostics, Raritan, NJ) and by recombinant immunoblot assay (RIBA) (Chiron, Emeryville, CA), and were tested for HCV RNA by polymerase chain reaction (PCR) [Shindo et al., 1991].

Samples investigated in this study were collected during medical follow-up and stored at −20°C until PCR analysis.

Viral RNA Preparation and PCR Amplification

Viral RNA was extracted from 100 μl of serum in the presence of silica and guanidinium thiocyanate according to the method described by Boom et al. [1990].

HCV RNA was detected by a nested PCR with two sets of primers specific for the 5′ untranslated region of the HCV genome [Shindo et al., 1991]. PCR products were analyzed by electrophoresis in a 3% agarose gel containing ethidium bromide and visualized under UV light.

For the detection of GBV-C/HGV, cDNA synthesis was carried out using random hexamers (Promega, Madison, WI) and AMV reverse transcriptase (Promega) according to the manufacturer's instructions.

The cDNA was amplified by a one-step PCR using primers of the 5′ non-coding region (Boehringer Mannheim, unpublished data) and NS5a region of GBV-C/HGV [Linnen et al., 1996]. PCR involved 45 cycles at 94°C for 15 sec, 55°C for 30 sec, and 72°C for 30 sec, followed by a single extension step of 72°C for 10 min.

For the amplification of sequences derived from the helicase (NS3) region of GBV-C/HGV, degenerate primers were used in a semi-nested PCR described by Yoshiba et al. [1995].

The PCR products of the 5′ non-coding region (180 bp), NS3 region (140 bp), and NS5a region (150 bp) were detected as described above.

Water and one sample of a negative control serum, extracted in parallel, were always used as control.

Determination of HCV Genotypes by Restriction Fragment Length Polymorphism (RFLP)

Genotyping was carried out in the 5′ non-coding region of the HCV genome using the technique described by Nakao et al. [1991] and Davidson et al. [1995] with slight modifications. DNA fragments were separated by polyacrylamide gel electrophoresis and visualized under UV light after ethidium bromide staining.

Statistical Analysis

Statistical analysis was carried out using the χ^2 test. P values less than 0.01 were considered statistically significant.

RESULTS

Sera from 100 HCV RNA-positive patients were examined for the presence of GBV-C/HGV by reverse transcription (RT)-PCR. PCR results were obtained from three different regions of the GBV-C/HGV genome (5′ non-coding, NS3, and NS5a regions) (Fig. 1). Nineteen

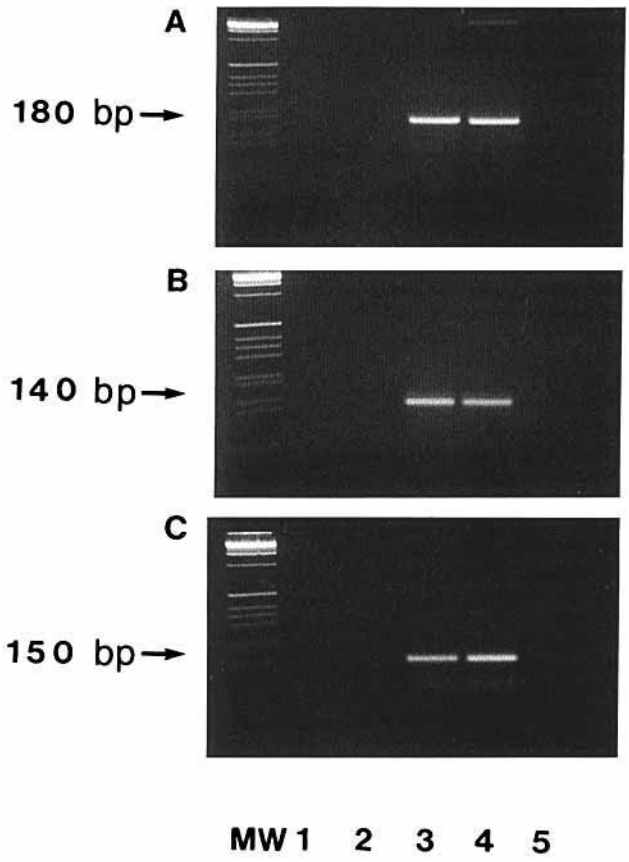

Fig. 1. PCR amplification of GBV-C/HGV RNA in patients coinfected with hepatitis C virus using primers directed to the 5′ non-coding **(A)**, the NS3 **(B)**, and the NS5a **(C)** regions of GBV-C/HGV. Lane 1: Extraction control (negative control serum). Lane 2: PCR control (H_2O). Lanes 3 and 4: HCV RNA-positive patients coinfected with GBV-C/HGV. Lane 5: HCV-positive but GBV-C/HGV-negative patient. MW: molecular weight marker.

patients (19%), 12 male and seven female, were found to be GBV-C/HGV RNA-positive, reacting with all primer sets used.

Analysis of GBV-C/HGV prevalence rates in chronic hepatitis C patients revealed that dual infections are related to shared risk factors. GBV-C/HGV-positive patients clustered among two subgroups: in the group of intravenous drug users 47% (9/19) of those with HCV were also infected with GBV- C/HGV and in the group of patients who underwent multiple transfusions 32% (6/19) were also positive for GBV-C/HGV RNA. The risk factors of the remaining patients were hemodialysis, 5% (1/19); hemophilia, 5% (1/19); or could not be identified (Table I).

Patients with both HCV and GBV-C/HGV had a mean age of 35 years (±10.5 years). The mean age of patients infected solely by HCV (n = 81) was 43 years (±14.6 years), the difference being statistically significant ($P < 0.01$). The considerably lower mean age of patients with dual infections is possibly explained by the fact that

TABLE I. Risk Category and Frequency of GBV-C/HGV RNA in Patients With Chronic Hepatitis C and Distribution of HCV Genotypes in These Groups

Risk category	GBV-C/HGV frequency	%	HCV genotypes	Frequency
Intravenous drug use	9/19	47%	1a	4/9
			1b	2/9
			3	3/9
Multiple transfusions	6/19	32%	1b	6/6
Hemodialysis	1/19	5%	1b	1/1
Hemophilia	1/19	5%	1a	1/1
Unknown	2/19	11%	1a	1/2
			4	1/2

a high percentage of these patients were intravenous drug users.

Determination of HCV genotypes in GBV-C/HGV coinfected patients by RFLP analysis showed that there was no association or correlation between the prevalence of GBV-C/HGV and any specific HCV genotype.

HCV genotype 1a was found in six samples (32%), genotype 1b was found in nine samples (47%), and genotype 3 was found in three samples (16%). Genotype 4 could be detected in one patient (5%) (Table I).

DISCUSSION

The high prevalence of GBV-C/HGV in chronic hepatitis C patients with related risk factors, such as intravenous drug abuse or multiple transfusions, indicates that GBV-C/HGV is transmitted efficiently parenterally and is frequently linked to hepatitis C infection.

Whether this is simply explained by similar modes of transmission or whether the HCV might have any influence—e.g., on the persistence or replication efficiency of GBV-C/HGV—remains to be determined. While clear disease manifestations of GBV-C/HGV have still to be examined, the detection of GBV-C/HGV in a group of patients with chronic liver disease raises the question of whether this may lead to a significant increase in the severity of clinical symptoms than would be the case in an exclusive infection with HCV or GBV-C/HGV.

The fact that clinically healthy carriers of GBV-C/HGV exist [Linnen et al., 1996] is evidence of the substantial health hazards posed by routine blood transfusion as not a single antibody test for GBV-C/HGV is yet available. The development of such test systems is a matter of utmost urgency since mass screening of blood donors, using the PCR technique, is highly impractical at present.

Antibody screening, therefore, remains the method of choice. Testing for HGV in blood or blood products must then be relegated high priority in furthering the aim of risk reduction in posttransfusion hepatitis.

ACKNOWLEDGMENTS

We thank Prof. K. Dietz, University of Tübingen, for statistical analysis of our results.

REFERENCES

Alter HJ, Purcell RH, Shih JW, Melpolder JC, Houghton M, Choo Q, Kuo G (1989): Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. New England Journal of Medicine 321:1494–1500.

Alter MJ, Margolis HS, Krawczynski K, Judson FN, Mares A, Alexander WJ, Hu PY, Miller JK, Gerber MA, Sampliner RE, Meeks EL, Beach MJ (1992): The natural history of community-acquired hepatitis C in the United States. New England Journal of Medicine 327:1899–1905.

Boom R, Sol CA, Salimans MM, Jansen CL, Wertheim-van Dillen PM, van den Noordaa J (1990): Rapid and simple method for purification of nucleic acids. Journal of Clinical Microbiology 28:495–503.

Davidson F, Simmonds P, Ferguson JC, Jarvis LM, Dow BC, Follett EA, Seed CR, Krusius T, Lin C, Medgyesi GA, Kiyokawa H, Olim G, Duraisamy G, Cuypers T, Saeed AA, Teo D, Conradie J, Kew MC, Lin M, Nuchaprayoon C, Ndimbie OK, Yap PL (1995): Survey of major genotypes and subtypes using RFLP of sequences amplified from the 5′ non-coding region. Journal of General Virology 76:1197–1204.

Leary TP, Muerhoff AS, Simons JN, Pilot-Matias TJ, Erker JC, Chalmers ML, Schlauder GG, Dawson GJ, Desai SM, Mushahwar IK (1996): Sequence and genomic organization of GBV-C: A novel member of the Flaviviridae associated with human non-A-E hepatitis. Journal of Medical Virology 48:60–67.

Linnen J, Wages J, Zhang-Keck ZY, Fry KE, Krawczynski KZ, Alter H, Koonin E, Gallagher M, Alter M, Hadziyannis S, Karayiannis P, Fung K, Nakatsuji Y, Shih J, Young L, Piatak M, Hoover C, Fernandez J, Chen S, Zou J-C, Morris T, Hyams KC, Ismay S, Lifson JD, Hess G, Foung SK, Thomas H, Bradley D, Margolis H, Kim JP (1996): Molecular cloning and disease association of hepatitis G virus: A transfusion transmissible agent. Science 271:505–508.

Muerhoff AS, Leary TP, Simons JN, Pilot-Matis TJ, Dawson GJ, Erker JC, Chalmers ML, Schlauder GG, Desai SM, Mushahwar IK (1995): Genomic organization of GB viruses A and B: Two new members of the Flaviviridae associated with GB agent hepatitis. Journal of Virology 69:5621–5630.

Nakao T, Enomoto N, Takada N, Takada A, Date T (1991): Typing of hepatitis C virus genomes by restriction fragment length polymorphism. Journal of General Virology 72:2105–2112.

Nübling CM, Löwer J (1996) GB-C genomes in a high-risk group, in plasma pools, and in intravenous immunoglobin. Lancet 347:68.

Peters T, Schlayer HJ, Preisler S, Kopp B, Berthold H, Gerok W, Rasenack J (1993): Frequency of hepatitis C in acute post-transfusion hepatitis after open-heart surgery: A prospective study in 1,476 patients. Journal of Medical Virology 39:139–145.

Shindo M, Di Bisegli AM, Cheung L, Shih JW, Cristiano K, Feinstone SM, Hoofnagle JH (1991): Decrease in serum hepatitis C viral RNA during alpha-interferon therapy for chronic hepatitis C. Annals of Internal Medicine 115:700–704.

Simons JN, Pilot-Matias TJ, Leary TP, Dawson GJ, Desai SM, Schlauder GG, Muerhoff AS, Erker JC, Buijk SL, Chalmers ML, Van Sant CL, Mushahwar IK (1995a): Identification of two flavivirus-like genomes in the GB hepatitis agent. Proceedings of the National Academy of Sciences USA 92:3401–3405.

Simons JN, Leary TP, Dawson GJ, Pilot-Matias TJ, Muerhoff AS, Schlauder GG, Desai SM, Mushahwar IK (1995b): Isolation of novel virus-like sequences associated with human hepatitis. Nature Medicine 1:564–569.

Thiers V, Lunel F, Valla D, Azar N, Fretz C, Frangeul L, Huraux

J-M, Opolon P, Brechot C (1993): Post- transfusional anti-HCV-negative non-A non-B hepatitis (II) serological and polymerase chain reaction analysis for hepatitis C and hepatitis B viruses. Journal of Hepatology 18:34–39.

Yoshiba M, Okamoto H, Mishiro S (1995): Detection of the GBV-C hepatitis virus in serum from patients with fulminant hepatitis of unknown origin. Lancet 346:1131–1132.

Zuckerman AJ (1996): Alphabet of hepatitis viruses. Lancet 347:558–559.